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REACTOGENICITY AND EFFECTIVENESS OF AEROGENIC VACCINATION AGAINST CERTAIN ZOONOSES

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In the present article the results are being presented of acute experiments on experimental animals as well as tests on people of the reactogenicity [ability to produce reactions] and immunological effectiveness of aerogenic vaccines against certain zoonoses (plague, tularemia, brucellosis, anthrax).

For the purpose of preparing the dry aerogenic vaccines live vaccinal strains were used: anthrax "STI", plague 1-17, brucellosis "VA" and tularemia No 15 (restored), which have been accepted in the Soviet Union for extensive use in subcutaneous, intracutaneous and percutaneous vaccination.

The experimental part of the work was conducted on guinea pigs, rabbits and sheep. Guinea pigs and rabbits were vaccinated in chambers of 0.5-1.5 cubic meter volume; the sheep, in wooden cages of three to five cubic meter volume. The immunization was carried out by the method of spraying three to 10 grams of aerogenic vaccines containing tens or hundreds of billions of living microbes per gram. The exposure time was 30-60 minutes. During the immunization the animals inhaled from several hundred thousand to tens of millions of living microbes.

For the purpose of obtaining comparative data, subcutaneous and percutaneous immunizations of experimental animals were also performed with the corresponding live vaccines. Aerogenic as well as subcutaneous vaccination was performed only once. Thirty days after the vaccination the animals were infected with the respective virulent cultures.

Guinea pigs immunized with dry, anti-plague aerogenic vaccine, survived in 60-80 percent of cases after subcutaneous and aerogenic infection with a culture of P. pestis in doses of 20-200 MLD. Guinea pigs which were immunized subcutaneously with live anti-plague vaccine using the same doses and methods of infection, survived in 75-100 percent

of the cases. The control, unvaccinated animals died in 100 percent of the cases in these experiments.

Similar results were obtained also in testing aerogenic vaccine against tularemia. Guinea pigs immunized with dry, aerogenic tularemia vaccine survived in 67-80 percent of cases after the subcutaneous or aerogenic infection with a virulent B. tularensis culture in doses of 100-1000 MLD. The guinea pigs which were immunized subcutaneously with live tularemia vaccine survived in 88-100 percent of cases; the unvaccinated control animals died in 100 percent of cases.

Aerogenic vaccine against brucellosis was also tested on guinea pigs. It was established that in 80-90 percent of the guinea pigs immunized with dry, aerogenic brucellosis vaccine, no generalized infectious process occurs, as a rule, after the subcutaneous infection of them with B. melitensis cultures in doses of five to 10 ID [infective doses]. The same results were obtained in guinea pigs immunized subcutaneously with live brucellosis vaccine. In control animals a generalized infection was recorded in 83-90 percent.

Somewhat different data were obtained in aerogenic infection of immunized guinea pigs with a B. melitensis culture in a dose of 100 ID. In the given case, a generalized infectious process developed in 51-53 percent of the guinea pigs.

The experiments performed showed that the aerogenic immunization against plague, tularemia and brucellosis in the guinea pigs creates an immunity of quite a high degree. However, the accumulated material (more than 1000 guinea pigs and 500 rabbits) at the same time attests to the fact that small laboratory animals are not very suitable models for testing the effectiveness of the dry aerogenic vaccines. Guinea pigs and rabbits cannot aspirate the necessary immunizing dose of aerogenic vaccines because of the anatomico-physiological characteristics of their respiratory apparatus (narrow cross-section of respiratory passages, small minute volume and superficial type of respiration). When large animals (sheep) were used for the work, the aerogenic method of immunization proved to be much more effective, as was expected. In sheep immunized with brucellosis dry aerogenic vaccine, no generalized infectious process was found, as a rule, following the subcutaneous, intracutaneous, intranasal or conjunctival

methods of infection with a B. melitensis culture.

The effectiveness of dry, aerogenic anthrax vaccine was studied most completely in comparative experiments with subcutaneous and percutaneous immunization with the "STI" vaccine. In all, 321 sheep were used in the testing. At various periods of time after the vaccination (from five days to seven months) subcutaneous, intracutaneous and aerogenic infection of the animals was carried with antrax spores in doses of 1000-10,000 MLD. Of 117 sheep immunized with dry, aerogenic anthrax vaccine, four died after the infection, that is, 3.3 percent. Of 80 sheep immunized subcutaneously with "STI" vaccine, four died, or five percent; of 49 sheep immunized percutaneously, seven died, or 16.3 percent. In the group of control animals 55 of 75 sheep died, or 73.3 percent.

Thus, in sheep it is possible to create a very strong immunity with anthrax aerogenic vaccine, which immunity does not lag behind that following subcutaneous vaccination and is superior to the immunity created following percutaneous immunization with the "STI" vaccine.

Along with the study of the effectiveness of the aerogenic vaccines in acute experiments, pathological examinations of the organs of the immunized animals were performed for the purpose of determining the reactogenicity of the preparations. In all, 315 guinea pigs and mice were used in the experiments. For the purpose of studying the dynamics of development of pathological processes the animals were sacrificed after periods of from five to 120 days at intervals of one to ten days. Changes which developed in the animals after aerogenic immunization were compared in each series of experiments with data obtained in the examination of animals subjected to subcutaneous immunization with analogous live vaccines.

An analysis of the results obtained permitted us to conclude that after aerogenic immunization the character of the histopathological changes found in the organs of the experimental animals were in general similar to the picture observed after subcutaneous vaccination (the pathological examinations were carried out by G. Ya. Gordon).

Positive results obtained in the study of aerogenic vaccines experimentally made it possible for us in 1957 to proceed with the testing of these preparations on limited groups of people. During the period 1957-1958, 487

persons were immunized by aerogenic vaccines against plague, tularemia, brucellosis and anthrax. (A. F. Shpigunov, A. A. Il'chenko, A. N. Malyshev, T. A. Levitov, B. S. Korostovtsev, A. B. Onikiyenko, N. K. Muratkhodzhayev, V. I. Dedyukina, O. V. Obukhova, as well as students of the Academy took an active part in the work on the study of the reactogenicity and immunological effectiveness of aerogenic vaccines carried out in the Military Medical Order of Lenin Academy imeni Kirov under the guidance of Professors I. I. Rogozin, P. A. Alisov, and A. A. Sinitskiy).

For the purpose of obtaining comparative data, 63 persons were immunized, in the capacity of controls, with live vaccines against plague, tularemia and anthrax by the percutaneous method; 25 persons were vaccinated subcutaneously against brucellosis.

Before immunization, all the participants of the experiments were checked in the dispensary, including a medical examination, clinical analyses of blood, sputum and urine as well as X-Ray examination of the organs of the chest cage. All persons inoculated against tularemia and brucellosis were first checked for the presence of immunological reorganization by means of agglutination reactions, the opsonophagocytic reaction (brucellosis) and appropriate allergic tests, and in those immunized against plague the complement fixation reaction was performed.

Medical Observation of the condition of health of the persons inoculated was established immediately after the time of immunization and was continued for five to seven to ten days (vaccination against plague and anthrax), 12-21 days (vaccination against tularemia and brucellosis). In addition to daily thermometry, an X-Ray examination of the organs of the chest cage was carried out on the first, third-fourth, seventh, 14th and 21st days of observation in those vaccinated, and clinical analyses of blood and sputum were also made. In accordance with the generally accepted methods of evaluation of the reactogenicity of vaccines, the reaction of the organism to the inoculation was subdivided into general and local. After aerogenic immunization the following were the signs of a general reaction: increase in body temperature, disturbance of the feeling of well being (signs of intoxication) in the form of headache, malaise, weakness, pains in the muscles and joints, etc., as well as changes in the hemogram; among the signs of a local reaction, changes in the lungs, respiratory passages and regional lymph glands.

The immunological effectiveness of the vaccines was determined by vaccination against brucellosis and tularemia by means of agglutination reactions, the opsonoph gocytic reaction (brucellosis) and allergic tests, and in the case of vaccination against plague the complement fixation reaction was performed. The performance of the reactions was arranged for seven, 15, 30, 90 and 180 days after the inoculation. The immunological effectiveness of the vaccines against anthrax was not determined in view of the absence of reliable tests.

Immunization of people was carried out in special cubicles 3.8 cubic meters and 10 cubic meters in volume as well as in a tent 12 cubic meters in volume. Before the experiment the number of live microbes per gram of dry vaccine was determined. The vaccines were released in the cubicles and in the tent by means of a sprayer; the exposure time was 15 minutes. During the immunization air samples were taken for the purpose of determining the number of live microbes per liter of air in the cubicle (or tent). By knowing the concentration of microbes, exposure time of vaccination and the respiratory volume of the person per minute, it is possible to determine the dose of vaccine inhaled, which is equal to the product of the figures mentioned.

In the immunization of 105 persons with aerogenic vaccine against brucellosis used in the proper dosage (one billion live microbes according to computation), a general reaction was noted in one person. It occurred in a moderately severe form and underwent complete resolution in three days. The reaction developed eight to 12 hours after the vaccination and manifested itself in a temperature rise to 38-38.5° and signs of intoxication (headache, rheumatic pains in the muscles and joints, weakness, loss of appetite). There were no local reactions found; no changes were found on the part of the lungs and the regional lymphatic nodes, according to the data of clinical and X-Ray examination. In the control group of 25 persons inoculated subcutaneously, general reactions occurred in nine persons, whereby in four they occurred in a moderately severe form.

The immunological effectiveness of aerogenic immunization against brucellosis was studied in 105 persons.

The results of the tests performed are presented in Table 1 (Page 37).

The Table shows that as early as seven days after the aerogenic immunization certain shifts are observed in the immunological figures. After 15 days, a positive Wright reaction is found in almost all those immunized, whereby the titers of it reach 1:1280, 1:5120; the Burnet reaction at this time proved to be positive in 70 percent. After 30 days, the Wright and Burnet reactions attained their maximal figures and were positive in 96 percent. Ninety days after the vaccination the immunological reactions continued to be recorded in 90 percent. In the group of those immunized subcutaneously, 15 and 30 days after the vaccination the Wright reaction was positive in titers lower than in the group of those immunized aerogenically, while the Burnet reaction at 30 days was positive in only 45 percent.

Table 1

Method of vaccination	No of persons examined	Times of examination (days)	No of persons with positive Burnet reaction	Number of persons with positive Wright reaction Total	1:20
Aerogenic	21	7	4	4	--
Subcutaneous	23	15	not performed	23	1
Aerogenic	42	15	30	40	--
Subcutaneous	22	30	10	22	1
Aerogenic	50	30	48	48	3
"	42	90	not performed	36	4
"	19	90	16		

Table 1 [continued]

Method of vaccination	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	1:5120
Aerogenic	3	1	--	--	--	--	--	--
Subcutaneous	4	6	7	3	1	1	--	--
Aerogenic	4	7	8	6	9	5	--	1
Subcutaneous	4	3	8	1	5	--	--	--
Aerogenic	5	8	15	9	6	1	1	--
"	14	5	10	3	--	--	--	--
"	not performed							

The figures of the osonophagocytic indices in aerogenically as well as cutaneously vaccinated persons attest to the high degree of immunological reorganization of the body. This material is presented in Table 2 (data of A. F. Shpigunov)

Table 2

Time of examination	Aerogenic vaccination		
	No of subjects	Opsono-phagocytic index	
		Average figures	maximal minimal
Before vaccination..	3	9.7	22/2
At Seven days. . . .	19	21.7	27/14
At 15 days.	41	26.0	31/16
At 30 days.	48	20.6	29/12
At 90 days.	41	24.3	37/14

Table 2 [continued]

Time of examination	Subcutaneous vaccination		
	No of subjects	Opsono-phagocytic index	
		Average figures	maximal minimal
Before vaccination. . .	35	14.4	23/4
At Seven days.	--	--	--
At 15 days	23	22.2	33/13
At 30 days	23	25.4	31/20
At 90 days	--	--	--

After the immunization of 128 persons with aerogenic vaccine against tularemia, which was used in a dose of 750,000 live microbes (according to the computation), a general moderately severe reaction was noted in two persons, and progressed just like the reaction to the vaccination against brucellosis. In one of them it was accompanied by an insignificant local reaction in the form of a rapidly

progressive bronchitis. After three days these signs completely disappeared. In the control group of 21 persons who were inoculated percutaneously, there were no general reactions observed; however, local reactions occurred in the majority of those immunized.

The immunological changes appear as early as a week after the vaccination. Over the course of 30 days there occurs a regular increase in the agglutinin titers in all those immunized and a regular increase in the sensitivity to the allergen; the agglutination reaction titer reaches 1:2560, and the allergic test becomes positive in 96 percent of those examined. In persons immunized percutaneously, the agglutinin titers were somewhat lower, and the allergic test proved to be positive in 95 percent.

The reactogenicity of anti-plague aerogenic vaccine was studied in 54 subjects. The vaccine used in proper dosages (750,000 live microbes according to the computation) which protected experimental animals from aerogenic infection produced a single general reaction of moderate severity, which, in general, progressed according to the clinical form described above; no local reaction was noted. In the control group of 17 persons inoculated percutaneously general reactions were observed in six; local reactions in all 17 persons. In part of the people immunized against plague a complement fixation reaction was performed 15 days after the vaccination (data of I. I. Daal'-Berg). In persons immunized aerogenically it proved to be positive, whereas in the control group, which had been immunized percutaneously, the reaction remained negative.

The reactogenicity of aerogenic vaccine against anthrax was studied in 32 persons; the immunizing dose was found to be within limits of 75,000,000 to 750,000,000 live microbes (by computation). There was no clinically expressed reaction in any of the subjects. In the control group, which consisted of 21 persons, who were inoculated percutaneously, there were no reactions either. In view of the absence of reliable tests, the evaluation of the immunological effectiveness of the vaccine against anthrax was not performed.

CONCLUSIONS

1. Dry aerogenic vaccines against plague, tularemia, brucellosis and anthrax are highly effective in acute

experiments on experimental animals.

2. Dry, aerogenic vaccines against plague, tularemia, brucellosis and anthrax when used in the proper dosages, are practically without reactions.

3. Aerogenic immunization against brucellosis, tularemia and plague assures a definite immunological reorganization of the bodies of people, recordable with agglutination reactions (tularemia, brucellosis) opsono-phagocytic reactions (brucellosis), allergic tests (tularemia, brucellosis) and complement fixation reaction (plague).

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